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Case Study: Pseudomonas aeruginosa Blood Stream Infection showing Derepressed AmpC β-lactamase Hyper-producing Mutants

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Abstract

Here we describe a middle aged-woman who had septic shock caused by a strain of *Pseudomonas aeruginosa* carrying derepressed AmpC hyper-producing genes. This was brought into view when the strain located in pipracillin/tazobactam area was exposed on agar plate to imipenem by diffusion causing "D-phenomena". Clinicians and microbiologists have to be aware of this phenomenon for a better choice of antimicrobials in treating their patients.

Keywords: Pseudomonas aeruginosa, Derepressed AmpC β-lactamase, Septic shock, chromosomally mediated, D-phenomena.

Introduction

Pseudomonas aeruginosa is a ubiquitous nosocomial pathogen affecting compromised hosts. It causes different infections that occur mostly in intensive care units, such as ventilator-associated pneumonia (VAP), surgical site infections (SSI), urinary tract infections (UTI) and blood stream infections (BSI); also it may cause nosocomial outbreaks with multiple sites infections (1, 2). Rarely does it cause community-acquired infections in adult and pediatric populations such as community-acquired pneumonia, blood stream infections, urinary tract infections, and skin-and-skin structures infections (3, 4, 5, 6). Treating *P. aeruginosa* infections may be problematic as it may adopt one or combination of several mechanisms of resistance against several antimicrobials, including intrinsic and acquired ones; such adoption made the pathogen able to deactivate some antimicrobials leading to therapeutic failures. Among resistance mechanisms that *P. aeruginosa* adopts are efflux pumps, Opr D porin, ESBL, metallobeta-lactamases and class 1 AmpC β-lactamase, the later may be expressed at low levels and may be hyper-produced (7, 8, 9, 10). Hyper-production of class 1 AmpC β-lactamase by *P. aeruginosa* disqualifies some useful antimicrobials employed for use in the treatment of serious infections (1, 9).

Here we discuss a case of an intractable shock in a young woman. Surgical site and blood cultures repeatedly grew *P. aeruginosa.* Moreover, it showed inducible resistant mutants to pipracillin/tazobactam on agar plate when it was placed in proximity to imipenem, this appeared as straightening of the clear zone in pipracillin/tazobactam zone facing imipenem and appearing as D-phenomena (**Figure 1**).

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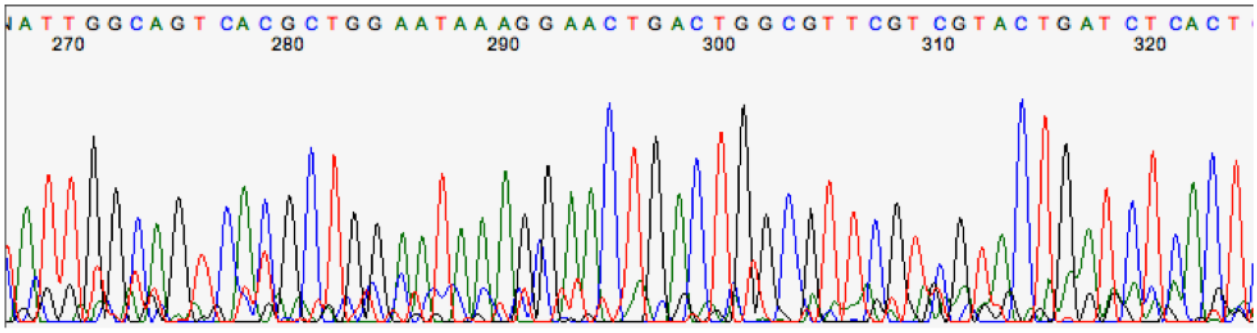

Figure 1. Sequence of the blood-isolated Pseudomonas aeruginosa. It showed a unique sequence which was not found in the genetic bank found at: http://www.pseudomonas.com/

Strain name: Pseudomonas aeruginosa PA7, g.5505076C>T, p.Asp237Asn Missense GAC(Asp) -> AAC(Asn)
Reference: gctggtgctggatctctagt C ggcggcgacgtcgccgtgaa
Query (isolated Pseudomonas): gctgctgctggatctctagt T cgcagaggtgtcgccgtgga
Protein changes as a phenotype that lead to the inducible resistance is: p.Ala235Ser

Case

A 51 years old Jordanian woman with past medical history of hypertension, she was admitted in a private Hospital, Amman - Jordan in January 2012 as she presented to the surgical department for hernia repair and abdominoplasty. On her first postoperative day she collapsed and coded; CPR was done for 15 minutes and was transferred to the ICU for mechanical ventilation, later noted to have suffered from brain damage, and she remained in hospital few months. During her prolonged hospital stay, earlier to her demise, she developed an intractable shock. She ran a stormy course with poor Glasgow scale, ABGs showed acidosis; pH < 6.9, pCO2 68 mmHg, pO2 67mmHg. Hemoglobin was 13.2 gm/dl, dropped to 5.4 gm/dl. Brain MRI showed acute infarction in basal ganglia, brain stem, thalami, posterior limbs of internal capsule and occipital lobes. Later, a follow up brain MRI showed massive brain edema and hemorrhages, and substantial atrophy in both hemispheres. Endoscopy showed no G.I. hemorrhage, thirteen units of packed RBCs and seven units of fresh frozen plasma were administered. Surgical site and blood cultures repeatedly grew *P. aeruginosa*. In addition, pus culture from the surgical site grew MRSA and *Acinetobacter* species. She was treated with several antipseudomonal agents including meropenem and pipracillin/tazobactam. The patient suffered from intractable shock, disseminated intravascular coagulation, multi-organ dysfunction, anoxic encephalopathy and died.

Microbiology and molecular biological isolation of *Pseudomonas aeruginosa*

Blood was cultured in Vitack II ARD blood culture bottles (BioMérieux SA. F-69280 Marcy l'Etoile, France) then spread on different plates (as in pus) on blood-agar, chocolate-agar, Mac-Conkey agar and SAB-agar plates, plates were incubated in aerobic and anaerobic environment, and incubated in thioglycolate for growth augmentation, and kept up to five days. Chocolate agar plates were incubated in CO_2 environment for twenty-four hours. Microorganisms were identified by colony morphology (shape, color, size) and gram stain, gram-negative bacteria grew on selective agar plate were lactose fermenter (LF) or non-fermenter (NLF) and processed by the automated Viteck II (BioMérieux SA. F-69280 Marcy l'Etoile, France). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 25423 were used as Controls. Kirby-Bauer antibiotic disk diffusion used to estimate sensitivity to that particular antibiotic using standard inoculum. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of the tested antibiotic.

Blood-isolate of *P. aeruginosa* grown on Muller Hinton agar showed D-phenomena by placing imipenem disk next to pipracillin/ tazobactam disk, its diffusion toward pipracillin/tazobactam zone induced hyper-production of resistant mutants, usually chromosomally mediated derepressed mutants that carry AmpC β-lactamase (11, 12).

Figure 1 showed that *P. aeruginosa* strain isolated from our case carry an unreported genome sequence when compared with gene bank.

DNA Isolation and Amplification

DNA was isolated from cultivated bacteria using the phenol/chloroform extraction technique. PCR amplification was done using specific Primers for *P. aeruginosa*. PCR Reaction mixtures (100 µl) were as follows:-10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl2, 200 µM (each) dATP, dCTP, dGTP

and dTTP; 1.25 U of Taq DNA polymerase (Amplitaq; Perkin Elmer), 0.1 µM of the each set of forward Primers and reverse primers which are specific for *P. aeruginosa*.
The reaction mixtures were subjected to the following empirically optimized thermal cycling parameters in a Perkin Elmer 2400 thermocycler following a "hot start": 96°C for 5 min followed by 40 cycles at 96°C for 1 min, 55°C for 1 min, 72°C for 1 min, followed by a final extension at 72°C for 10 min. *P. aeruginosa* ATCC 27853 DNA control and multiple negative controls were included in every set of PCR reactions.

Sequencing

Purification of PCR product was done using ExoSAP. Sequencing of the bacteria was conducted using Sanger method (big dye terminator reaction V3.0 (applied biosystems)) for sequencing of the forward and reverse primers, reading of the sequence done on ABI 310 genetic analyzer System. The subsequent sequence was compared to normal bacterial sequence found at gene bank website (http://www.pseudomonas.com/) with no previously matched sequence.

Discussion

A common mechanism for the development of resistance to penicillins like piperacillin and cephalosporins (mostly ceftazidime) is a selective mutation causing the hyper-production of the chromosomally mediated cephalosporinase AmpC. AmpC β-lactamase gene is present in most *Enterobacteriaceae*, *P. aeruginosa* and other non-fermenting gram-negative bacilli. AmpC is hyper-produced when suppression or inactivation of AmpD and AmpE occur. During treatment with β-lactams, resistant mutants showing constitutive high levels of AmpC production are frequently selected, leading to therapeutic failure which in turn prohibits the use of antimicrobial agents such as pipracillin/tazobactam and ceftazidime (1).

In our case *P. aeruginosa* showed a phenomenon of hyper-production of the chromosomally-mediated β-lactamase (AmpC), when it was exposed to a carbapenem (here meropenem). Meanwhile, *Pseudomonas aeruginosa* may acquire resistance to carbapenems by porin loss (OprD) and proteins involved in four efflux systems (mexA, mexC, mexE, and mexX) (9). Our patient suffered of intractable shock though she was on several β-lactam antimicrobials and later meropenem. During β-lactams and later carbapenems treatment, blood cultures revealed acquired inducible *P. aeruginosa* mutants carrying *AmpC* which lead to hyper-production of AmpC β-lactamase. This lead to treatment failure of the last (non carbapenem) β-lactams that were administered. Nearly before her demise meropenem was re-administered when mortality was looming.

This case rings a bell for infectious diseases practitioner and clinical microbiologists to suspect resistance mechanisms by being aware of the D-phenomena caused by AmpC β-lactamase hyper-production. When patients are initially exposed to a carbapenem as a therapeutic regimen for treating serious gram-negative infections, it may not be wise to change back to other β-lactams like ceftazidime or pipracillin/tazobactam, unless vigilance to pathogen diagnosis and susceptibility is sought. Even carbapenems may suffer from resistance based on the presence of AmpC and OprD (D2 porin protein) loss or efflux mechanisms (14). This pattern of resistance has to be communicated to other health-care providers; to be aware that carbapenems may select hyper-production of AmpC β-lactamase in gram-negative bacilli; therefore, other non-carbapenem β-lactams regimen cannot be an option.

As a reminder, a similar well known "D-phenomenon" occurs in *S. aureus* when Clindamycin disk is placed in proximity to Erythromycin disk on Muller Hinton agar. Here Erythromycin induces resistant mutants in the clindamycin zone (15, 16).

In conclusion, microbiologists and clinicians have to be aware of the possibility of AmpC β-lactamase hyper-production should a D-phenomena is detected by clinical laboratory; when patients have clinical deterioration, especially when carbapenem containing regimen preceded other β–lactams antimicrobials, as commonly practiced in intensive care units, where combinations and hopping to other antimicrobials frequently occur.

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